IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

John KENTEN, et al — Atty. Ref.: 2757-5

Serial No. Unassigned — Group:

Filed: June 14, 2001 — Examiner:

For: CONTROLLING PROTEIN LEVELS IN EUCARYOTIC ORGANISMS

* * * * * * * * * * *

June 14, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

FIRST PRELIMINARY AMENDMENT

Please amend this application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 17, line 8, with the following rewritten paragraph:

Since the method of the subject invention does not make use of the 'active' site of a given target protein, it is able to achieve a level of specificity for a drug molecule previously considered extremely difficult and uncertain using conventional drug discovery efforts. This advantage stems from the constraints placed on existing drug discovery efforts that are based on the need to inhibit an enzyme or receptor binding site that is common to a series of different proteins in different tissues and with very different roles in the physiology of the organism.

These constraints are based on the common structural elements in the binding or catalytic sites of these related proteins which form the site for conventional drug discovery. The common structural elements typically result in the selection of drugs that will inhibit the whole series of different proteins as these structural elements form the basis for the binding of the drug molecules selected from the screen. Thus conventional drug screening approaches result in the selection of drug hits which do not provide the degree of selectivity desired to bring about a desired therapeutic affect. In the subject invention, since the active site does not need to be the target for the selection of molecules that form the basis of the drug molecule, a significant improvement in the discovery of highly selective drugs is achieved. The consequence is the development of drugs with an enhanced therapeutic value. This advantage is further enhanced by the ability of this drug discovery approach to make use of the whole surface of the given protein target to find molecules with the desired binding specificity. This advantage is then combined with the ability to make use of a rapid screen that is wholly based on the use of binding and thus achieves a level of speed and through put not possible with other methods. This advantage is of great value when the desire is to find a very specific inhibitor of a given member of a protein family that is highly homologous and thus extremely difficult or impossible for drug discovery based on the effector, receptor or catalytic site of the given protein. This invention thus provides a means for the development of compounds of the invention which are

IN THE CLAIMS

Please cancel Claims 1-23, 31-35, 38, 39, 41 and 42 without prejudice.

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

24. (Amended) A method of reducing the level and/or activity of a target protein in an eukaryotic cell via the activation of ubiquitination of said target protein comprising contacting said cell with a compound comprising;

a) a ubiquitination recognition element which is able to bind to either the E3 or E2 elements of the ubiquitination system, wherein said ubiquitination recognition element has a molecular weight less than 30,000 and has a binding affinity for said E3 and/or E2 elements of the ubiquitination system of at least 10^2 M^{-1} and;

b) a target protein binding element that is able to bind specifically to said target protein wherein said target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for said target protein greater than 10^5 M^{-1},

wherein said ubiquitination recognition element is covalently linked to said target protein binding element.

25. (Amended) The method of claim 24 where said reduction causes a physiological or metabolic change.

26. (Amended) The method of claim 24 where said reduction causes a pharmacological change.

27. (Amended) The method of claim 24 where said reduction treats a disease.

29. (Amended) The method of claim 28 where said target protein is an antigen.

36. (Amended) A method of selectively targeting ubiquitination in a cell comprising contacting said cell with a compound comprising;

a ubiquitination recognition element which is able to bind to either the E3 or E2 functional elements of the ubiquitination system, wherein said ubiquitination recognition element has a molecular weight less than 30,000 and has a binding affinity for said E3 and/or E2 elements of the ubiquitination system of at least 10^2 M^{-1} and;

a target protein binding element that is able to bind specifically to a target protein wherein said target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for said target protein greater than 10^5 M^{-1},

wherein said ubiquitination recognition element is covalently linked to said target protein binding element.

REMARKS

Claims 24-30, 36, 37 and 40 are presented for examination.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made**."

Favorable action on this application is awaited.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

The paragraph beginning at page 17, line 8:

Since the method of the subject invention does not make use of the 'active' site of a given target protein, it is able to achieve a level of specificity for a drug molecule previously considered extremely difficult and uncertain using conventional drug discovery efforts. This advantage stems from the constraints placed on existing drug discovery efforts that are based on the need to inhibit an enzyme or receptor binding site that is common to a series of different proteins in different tissues and with very different roles in the physiology of the organism. These [constrains] constraints are based on the common structural elements in the binding or catalytic sites of these related proteins which form the site for conventional drug discovery. The common structural elements typically result in the selection of drugs that will inhibit the whole series of different proteins as these structural elements form the basis for the binding of the drug molecules selected from the screen. Thus conventional drug screening approaches result in the selection of drug hits which do not provide the degree of selectivity desired to bring about a desired therapeutic affect. In the subject invention, since the active site does not need to be the target for the selection of molecules that form the basis of the drug molecule, a significant improvement in the discovery of highly selective drugs is achieved. The consequence is the development of drugs with an enhanced therapeutic value. This advantage is further enhanced by the ability of this drug discovery approach to make use of the whole surface of the given protein target to find molecules with the desired binding specificity. This advantage is then combined with the ability to make use of a rapid screen that is wholly based on the use of binding and thus achieves a level of speed and through put not possible with other methods. This advantage is of great value when the desire is to find a very specific inhibitor of a given member of a protein

family that is highly homologous and thus extremely difficult or impossible for drug discovery based on the effector, receptor or catalytic site of the given protein. This invention thus provides a means for the development of compounds of the invention which are

IN THE CLAIMS

24. (Amended) A method of [modulating] reducing the level and/or activity of [at least one] a target protein in an eukaryotic cell via the [modulation] activation of ubiquitination of said [at least one] target protein comprising contacting said cell with a compound comprising;

c) a ubiquitination recognition element which is able to bind to either the E3 or E2 elements of the ubiquitination system, wherein said ubiquitination recognition element has a molecular weight less than 30,000 and has a binding affinity for said E3 and/or E2 elements of the ubiquitination system of at least 10^2 M^{-1} and;

d) a target protein binding element that is able to bind specifically to [a] said target protein wherein said target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for said target protein greater than 10^5 M^{-1},

wherein said ubiquitination recognition element is covalently linked to said target protein binding element.

25. (Amended) The method of claim 24 where said [at least one target protein is modulated to cause] reduction causes a physiological or metabolic change.

26. (Amended) The method of claim 24 where said [at least one target protein is modulated to cause] reduction causes a pharmacological change.

27. (Amended) The method of claim 24 where said [at least one target protein is modulated to treat] reduction treats a disease.

29. (Amended) The method of claim 28 where said [at least one] target protein is an antigen.

36. (Amended) A method of selectively targeting ubiquitination in a cell comprising contacting said cell with a compound [as in claim 1] comprising:

a ubiquitination recognition element which is able to bind to either the E3 or E2 functional elements of the ubiquitination system, wherein said ubiquitination recognition element has a molecular weight less than 30,000 and has a binding affinity for said E3 and/or E2 elements of the ubiquitination system of at least 10^2 M^{-1} and;

a target protein binding element that is able to bind specifically to a target protein wherein said target protein binding element has a molecular weight of

less than 30,000 and has a binding affinity for said target protein greater than 10^5 M^{-1},

wherein said ubiquitination recognition element is covalently linked to said target protein binding element.

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For: CONTROLLING PROTEIN LEVELS IN EUCARYOTIC ORGANISMS

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June 14, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

SECOND PRELIMINARY AMENDMENT

Please amend the above application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 33, line 16, with the following rewritten paragraph:

RAALGEIGN (SEQ ID NO 26),

RAVLEEIGN (SEQ ID NO 27),

RSAFGDITN (SEQ ID NO 28),

RSILGVIQS (SEQ ID NO 29),

RAALGVITN (SEQ ID NO 30),

RTVLGVIGDN (SEQ ID NO 31),

RTVGVLQEN (SEQ ID NO 32),

RAALGTVGE (SEQ ID NO 33),

RTVLGVLTEN (SEQ ID NO 34),

RAALAVLKSGN (SEQ ID NO 35),

RLPLAAKDN (SEQ ID NO 36),

RQLFPIPLN (SEQ ID NO 37),

RRTLKVIQP (SEQ ID NO 38),

expressed as a general structure

R(A/T)(A)LGX(I/V)(G/T)(N) (SEQ ID NO 39), or expressed as a consensus RXXLGXIXN (SEQ ID NO 53), where X is any amino acid and amino acids in parentheses occur in more than 50% of known destruction sequences.

Please replace the paragraph beginning at page 34, line 26, with the following rewritten paragraphs:

Some examples of ubiquitination recognition elements based on the N-recognin include;

Arg-∈Ahx-Cys

Arg-β-Ala-∈Ahx-Cys

Arg-∈Ahx-∈Ahx-Cys

Phe-∈Ahx-Cys

Phe-β-Ala-∈Ahx-Cys

Phe-∈Ahx-∈Ahx-Cys

Arg-Ala-∈Ahx-Cys

Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66)

Arg-Ala-∈Ahx-∈Ahx-Cys

Please replace the paragraph beginning at page 35, line 1, with the following rewritten paragraph:

Phe-Ala-∈Ahx-Cys

Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67)

Phe-Ala-∈Ahx-∈Ahx-Cys

Please replace the paragraph beginning at page 37, line 1, with the following rewritten paragraph:

R(A/T)(A)LGX(I/V)(G/T)(N) (SEQ ID NO 39), or expressed as a consensus RXXLGXIXN (SEQ ID NO 53), where X is any amino acid and amino acids in parentheses occur in more than 50% of known destruction sequences.

Please replace the paragraph beginning at page 58, line 1, with the following rewritten paragraph:

motif CCXXCC (SEQ ID NO:47) and WEAAAREACCRECCARA (SEQ ID NO 48), and AEAAAREACCRECCARA (SEQ ID NO 49), is 4',5'-bis(1,3,2-dithioarsolan-2-yl)fluorescein with other bis-organoarsenical being useful (Griffin BA, 1998, Science 218, 269, which is hereby incorporated by reference in its entirety).

Please replace the paragraph beginning at page 60, line 19, with the following rewritten paragraph:

Control of protein levels in the liver of a transgenic organism

An example of the above embodiment is the demonstration of targeted ubiquitination to mediate quantitative and tissue specific control of gene expression in transgenic mice. The expression vector was constructed using the luciferase gene and a liver specific promoter ~ the promoter of the liverenriched activator protein driving the expression of the luciferase gene (Kistner A., 1996, Proc. Natl. Acad. Sci. 93, 10933-10938). The luciferase gene was engineered to contain the AEAAAREACCRECCARA (SEQ ID NO 49), sequence at the C terminus using synthetic oligonucleotides and PCR based

Please replace the paragraph beginning at page 73, line 14, with the following rewritten paragraph:

Further ubiquitination recognition elements are synthesized as

follows using methods described above.

1. Arg-Ala-∈Ahx-Cys
2. Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66)
3. Arg-Ala-∈Ahx-∈Ahx-Cys
4. Phe-Ala-∈Ahx-Cys
5. Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67)
6. Phe-Ala-∈Ahx-∈Ahx-Cys

Please insert the attached paper copy of the sequence listing beginning at page 85 and renumber the claims pages accordingly.

IN THE CLAIMS

Please substitute the following amended claim for corresponding claim previously presented. A copy of the amended claim showing current revisions is attached.

13. (Amended) A compound as in claim 1 wherein said ubiquitination recognition element contains a moiety selected from the group consisting of Arg-∈Ahx-Cys, Arg-β-Ala-∈Ahx-Cys, Arg-∈Ahx-∈Ahx-Cys, Phe-∈Ahx-Cys, Phe-β-Ala-∈Ahx-Cys, Phe-∈Ahx-∈Ahx-Cys, Arg-Ala-∈Ahx-Cys, Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66), Arg-Ala-∈Ahx-∈Ahx-Cys, Phe-Ala-∈Ahx-Cys, Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67) and Phe-

Ala-∈Ahx-∈Ahx-Cys.

REMARKS

The above amendments have been made to place the application in a more traditional format.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made**."

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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Arlington, VA 22201-4714
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Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

The paragraph beginning at page 33, line 15:

R(A/T)(A)LGX(I/V)(G/T)(N) (SEQ ID NO 39), or expressed as a consensus RXXLGXIXN (SEQ ID NO [40] 53), where X is any amino acid and amino acids in parentheses occur in more than 50% of known destruction sequences.

The paragraph beginning at page 34, line 26:

Some examples of ubiquitination recognition elements based on the N-recognin include;

Arg-∈Ahx-Cys

Arg-β-Ala-∈Ahx-Cys

Arg-∈Ahx-∈Ahx-Cys

Phe-∈Ahx-.Cys

Phe-β-Ala-∈Ahx-Cys

Phe-∈Ahx-∈Ahx-Cys

Arg-Ala-∈Ahx-Cys

Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66)

Arg-Ala-∈Ahx-∈Ahx-Cys

The paragraph beginning at page 35, line 1:

Phe-Ala-∈Ahx-Cys

Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67)

Phe-Ala-∈Ahx-∈Ahx-Cys

The paragraph beginning at page 37, line 1:

R(A/T)(A)LGX(I/V)(G/T)(N) (SEQ ID NO 39), or expressed as a consensus RXXLGXIXN (SEQ ID NO [40] 53), where X is any amino acid and amino acids in parentheses occur in more than 50% of known destruction sequences.

The paragraph beginning at page 58, line 1:

motif CCXXCC (SEQ ID NO:47) and WEAAAREACCRECCARA (SEQ ID NO 48), and AEAAAREACCRECCARA (SEQ ID NO 49), is 4',5'-bis(1,3,2-dithioarsolan-2-yl)fluorescein with other bis-organoarsenical being useful (Griffin BA, 1998, Science 218, 269, which is hereby incorporated by reference in its entirety).

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2. Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66)
3. Arg-Ala-∈Ahx-∈Ahx-Cys
4. Phe-Ala-∈Ahx-Cys
5. Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67)
6. Phe-Ala-∈Ahx-∈Ahx-Cys

IN THE CLAIMS

13. (Amended) A compound as in claim 1 wherein said ubiquitination recognition element contains a moiety selected from the group consisting of Arg-∈Ahx-Cys, Arg-β-Ala-∈Ahx-Cys, Arg-∈Ahx-∈Ahx-Cys, Phe-∈Ahx-Cys, Phe-β-Ala-∈Ahx-Cys, Phe-∈Ahx-∈Ahx-Cys, Arg-Ala-∈Ahx-Cys, Arg-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:66), Arg-Ala-∈Ahx-∈Ahx-Cys, Phe-Ala-∈Ahx-Cys, Phe-Ala-β-Ala-∈Ahx-Cys (SEQ ID NO:67) and Phe-Ala-∈Ahx-∈Ahx-Cys.